National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1546

Meat Homogenate

Standard Reference Material (SRM) 1546 is intended primarily for use in validating methods for determining fatty acids, cholesterol, proximates, calories, vitamins, and minerals in canned meat products and similar materials. This SRM can also be used for quality assurance when assigning values to in-house control materials. The meat homogenate is a mixture of pork and chicken products blended together in a commercial process. A unit of SRM 1546 consists of four cans, each containing approximately 85 g of material.

Certified Concentration Values: Analyses for value assignment were performed by NIST and collaborating laboratories. All certified values are the equally weighted means of the measurements made by NIST and the grand-mean from the laboratories reporting results for a given analyte, and the associated uncertainties are expressed at the 95 % level of confidence [1,2]. Values are reported on an as-received (not dry-mass) basis in mass fraction units [3]. The certified concentration values of selected fatty acids and cholesterol in SRM 1546 are provided in Table 1 and certified concentration values for calcium, iron, and sodium are provided in Table 2.

Reference Concentration Values: Reference concentration values are provided for additional fatty acids (Table 3), proximates and calories (Table 4), water-soluble vitamins and sucrose (Table 5), and minerals and trace elements (Table 6). These reference concentrations were derived from results reported by collaborating laboratories. Reference values are provided for analytes for which (1) results have not been confirmed by an independent analytical technique as required for certification, (2) the disagreement among the methods was greater than expected for certified values, and/or (3) analyses have not been performed at NIST.

Information Concentration Values: Information concentration values for additional analytes are provided in Table 7. These concentrations are listed as information values because of the larger disagreement of results among laboratories and/or are derived from analyses performed by a limited number of the collaborating laboratories.

Expiration of Certification: The certification of this SRM is valid until **01 January 2004**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

Analytical measurements at NIST were performed by C.S. Phinney, L.T. Sniegoski, L.K. Walton, and L.J. Wood of the NIST Analytical Chemistry Division.

Coordination of the technical measurements leading to the certification of this SRM was performed by K.E. Sharpless and M.J.Welch of the NIST Analytical Chemistry Division.

The support aspects involved with the certification and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert.

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Certificate Issue Date: 10 May 1999

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SRM 1546 was developed at the request of the Food Safety Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA). Coordination between the FSIS and NIST was provided by G.V. Iyengar, consultant to the NIST Standard Reference Materials Program. Consultation on the acquisition of the base material was provided by W.R. Wolf of the USDA Beltsville Human Nutrition Research Center.

Statistical analysis was provided by L.M. Gill of the NIST Statistical Engineering Division.

NOTICE AND WARNING TO USERS

Storage: The SRM should be stored at room temperature or under refrigeration in the original unopened cans. The certification does not apply to contents of previously opened cans as the stability of all analytes has not been investigated.

Warning: For laboratory use only. Not for human consumption.

Instructions for Use: Before use, the contents of the can should be mixed by thorough stirring or mashing. One technique recommended is to transfer all of the contents of a can to a plastic bag and then manually squeezing the bag to blend the material. Care should be taken to avoid separating fat from the material. A minimum sample size of 1 g should be used for any analytical determination to be in accord with the uncertainties reported in this certificate.

SOURCE, PREPARATION AND ANALYSIS[1]

Source and Preparation: SRM 1546 is a mixture of pork, mechanically-separated chicken, ham, salt, sucrose, water, and spices and was prepared by the Hormel Foods Corporation, Austin, MN, by a commercial process that included cooking, grinding, blending, and sieving prior to canning under sterile conditions. A small quantity of sodium nitrite was added as a preservative prior to canning. The cans were sequentially numbered in the filling process to facilitate evaluation of homogeneity over the course of the filling run.

Analytical Approach: Analyses were performed by NIST and by collaborating laboratories. A stratified random sampling plan was devised for all of the analyses.

NIST Analyses for Cholesterol and Fatty Acids: Cholesterol was measured using the isotope dilution/gas chromatography/mass spectrometry (ID/GC/MS) method developed at NIST for serum cholesterol [3] and modified for the determination of cholesterol in food matrices using AOAC Method 43.235 for hydrolysis [4]. Three sets of samples were prepared. Each set consisted of duplicate samples from each of three cans of SRM 1546, one jar of SRM 1544 Fatty Acids and Cholesterol in a Frozen Diet Composite, and one jar of SRM 1845 Whole Egg Powder. The latter two materials were used as controls. Each can of SRM 1546 was opened, the contents were thoroughly stirred with a spatula, and two 1 g samples were withdrawn and accurately weighed into round-bottomed flasks. An aliquot of a solution containing a known mass of the internal standard, cholesterol-$^{13}C_3$, was added to each flask. Hydrolysis of cholesterol esters was accomplished by refluxing the samples in an alcohol-KOH solution for 1 h. Hexane was then used to extract the cholesterol. A portion of the hexane extract was evaporated to dryness and N,O-bis(trimethylsilyl)acetamide was added to convert cholesterol to its trimethylsilyl (TMS) derivative. Analyses were performed on a GC/MS system operated in the electron ionization mode with selected ion monitoring at m/z 458 and m/z 461 for the unlabeled and labeled cholesterol-TMS, respectively. The GC was equipped with a 30 m (5 % phenyl 95 % (mole fraction) methyl polysiloxane) non-polar fused silica column directly interfaced to the ion source. Standards consisting of mixtures of known quantities of pure unlabeled cholesterol (SRM 911b) and cholesterol-$^{13}C_3$ were run before and after the samples to generate composite linear regressions for calculation of the quantity of cholesterol in the samples.

Fatty acids (FAs) were also determined by ID/GC/MS. Three sets of samples were prepared. Each set consisted of duplicate samples from each of three cans of SRM 1546 and two jars of SRM 1544. Two solutions of deuterated fatty acids were prepared. One, containing major components, included C18:1 (oleic acid)-d_2, C16:0 (palmitic acid)-d_3, and C18:0 (stearic acid)-d_3. The minor component solution included C10:0 (capric acid)-d_3, C12:0 (lauric acid)-d_3, C14:0 (myristic acid)-d_3, and C20:0 (arachidic acid)-d_3. These labeled solutions were used

[1]Certain commercial materials and equipment are identified to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.

for the preparation of standards and for spiking of samples. Standards were prepared from solutions made from two different weighings of unlabeled FA standards (Nu-Chek-Prep, Inc., Elysian, MN), and the same solutions of deuterated FAs used for spiking of the samples.

Each can of meat homogenate was opened and the contents were mixed well in a plastic bag by squeezing repeatedly. Amounts of approximately 1 g were weighed accurately and combined with approximately 1.6 g of pre-cleaned diatomaceous earth (600 to 1400) μm and then loaded into a pressurized fluid extraction (PFE) cell. The cells were spiked with C13:0 triglyceride (tritridecanoin) and C19:0 triglyceride (trinonadecanoin) in chloroform as extraction recovery surrogates. The meat homogenate sample materials were subjected to semi-static PFE with hexane:dichloromethane:methanol (70:25:5) at 125 °C and 10,000 kPa (1500 psig) for 5 min. After PFE, the extracts were diluted to known volume (50 mL), and a 5 mL aliquot was spiked with deuterated internal standard mixture, allowed to equilibrate, and subjected to alkaline hydrolysis for 1 h in 1 mol/L sodium hydroxide solution at 60 °C. After hydrolysis, the samples were acidified with 1.0 mL of 6 mol/L HCl and buffered with 2.5 mL of pH 4 buffer. The FAs were subsequently extracted with three 5 mL portions of hexane. A 1.0 mL aliquot of this material was treated with 50 μL of 1,1-dimethoxytrimethylamine to form the corresponding fatty acid methyl esters (FAME). Analysis of the resultant FAMEs mixture was performed on an ion trap mass spectrometer. Separation was accomplished on a 30 m polyethylene glycol chromatographic column, followed by electron ionization and full-scan mass spectrometric detection.

NIST Analyses for Calcium, Iron, and Sodium: Two 3.5 g portions were taken from each of eight cans of SRM 1546 and from one can of Certified Reference Material (CRM): LGC 7002, Pork/Chicken Meat, Laboratory of the Government Chemist (LGC) Teddington, UK, as a control material. The samples and accompanying blanks were digested in 10 mL each of concentrated HNO_3 and $HClO_4$ at 160 °C until solutions were clear. The acids were evaporated and the residues redissolved in 10 mL water and 2 mL concentrated HNO_3 and transferred to 50 mL volumetric flasks with the addition of water. Separate aliquots from these solutions were taken for measurements of each element. Two aliquots were taken for each element from each solution, one of which was spiked with a known concentration of the element, and the aliquots diluted up to a final volume at a final acid concentration of 3.2 % HNO_3. Measurements were performed using inductively coupled plasma atomic emission spectrometry. Emission wavelengths monitored were: 393.366 nm (Ca), 238.204 nm (Fe), and 589.592 nm (Na). Each solution was measured four times and the results were averaged. Spike recoveries were measured to correct for matrix effects.

Collaborating Laboratories' Analyses: The National Food Processors Association (NFPA) Food Industry Analytical Chemists Subcommittee (FIACS) laboratories (Appendix A) were asked to use AOAC methods or their equivalents and to make single measurements from each of four cans. The laboratories listed in Appendix A also analyzed SRM 1544, SRM 1846 Infant Formula, and LGC CRM 7002 for quality assurance. A summary of the methodological information and the number of laboratories using a particular analytical technique is provided in Appendix B. Three laboratories not affiliated with the NFPA also performed analyses, two only for fat content and the third for minerals only. One laboratory reported total extractable fat by two methods: one method being a conventional Soxhlet extraction and the other a pressurized fluid extraction. The second laboratory performed fatty acid analysis after using a supercritical fluid extraction to isolate the fat containing fraction. The third laboratory measured a number of inorganic constituents using thermal neutron prompt gamma activation analysis.

Homogeneity Assessment: The homogeneity of cholesterol in 1 g and whole-can samples was assessed at NIST using the methods described. Statistically significant heterogeneity was found for this analyte at the 1 g level; and, therefore, a 3 % component for inhomogeneity has been added to the uncertainties for all analytes although the homogeneity of the other analytes was not assessed.

Value Assignment: The laboratories listed in Appendix A reported the individual results for each of their analyses for a given analyte. The mean of each laboratory's results was then determined. For calculation of assigned values for analytes that were measured only by the collaborating laboratories, each of the laboratory means was weighted equally. For analytes that were measured by both collaborating laboratories and NIST, the grand mean of the individual collaborating laboratory means was equally weighted with the mean from the NIST data.

Table 1. Certified Concentrations for Fatty Acids[a,b] and Cholesterol[b]

Constituent	Common Name	Mass Fraction (g/kg)
Decanoic Acid (C:10.0)	Capric Acid	0.171 ± 0.032
Dodecanoic Acid (C:12.0)	Lauric Acid	0.133 ± 0.028
Tetradecanoic Acid (C:14.0)	Myristic Acid	2.53 ± 0.19
Hexadecanoic Acid (C:16.0)	Palmitic Acid	45.6 ± 3.9
Octadecanoic Acid (C:18.0)	Stearic Acid	21.7 ± 2.9
(Z)-9-Octadecenoic Acid[c] (C:18.1)	Oleic Acid	82.0 ± 9.6
Eicosanoic Acid (C:20.0)	Arachidic Acid	0.315 ± 0.063
Cholesterol		0.750 ± 0.072

[a] Fatty acid concentrations are expressed as free fatty acids. To convert to the equivalent triglyceride or methyl ester (FAME) concentration, see Reference 5.

[b] Each certified concentration value, expressed as a mass fraction for the material as received, is an equally weighted mean from the combination of results from analyses by NIST and the grand mean of laboratories listed in Appendix A. The uncertainty in the certified concentration is calculated as $U = ku_c + B$. The quantity u_c is the combined standard uncertainty, calculated according to the ISO Guide [1], and accounts for the combined effect of the within variance for all participating laboratories at one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. B is a bias adjustment for the difference between methods, which is the maximum difference between the certified value and method means [2]. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendix B.

[c] This is the primary component contributing to the measured value. There may be low levels of other unsaturated fatty acids that coeluted with the principal component and contributed to the signal measured.

Table 2. Certified Concentrations for Selected Elements[a]

Constituent	Mass Fraction (mg/kg)
Calcium	323 ± 28
Iron	11.4 ± 1.0
Sodium	9990 ± 716

[a] Each certified concentration value, expressed as a mass fraction for the material as received, is an equally weighted mean from the combination of results from analyses by NIST and the grand mean of laboratories listed in Appendix A. The uncertainty in the certified concentration is calculated as $U = ku_c + B$. The quantity u_c is the combined standard uncertainty, calculated according to the ISO Guide [1], and accounts for the combined effect of the within variance for all participating laboratories at one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. B is a bias adjustment for the difference between methods, which is the maximum difference between the certified value and method means [2]. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendix B.

Table 3. Reference Concentrations for Selected Fatty Acids[a,b]

NOTE: These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification and/or analyses have not been performed at NIST.

Constituent	Common Name	Mass Fraction (g/kg)
Octanoic Acid (C8:0)	Caprylic Acid	0.024 ± 0.013
9-Hexadecenoic Acid[c] (C16:1)	Palmitoleic Acid	6.83 ± 0.66
9,12-Octadecadienoic Acid[c] (C18:2)	Linoleic Acid	19.6 ± 2.0
9,12,15-Octadecatrienoic Acid[c] (C18:3)	Linolenic Acid	1.41 ± 0.35
11-Eicosenoic Acid[c]	Eicosenoic Acid	1.56 ± 0.23
5,8,1,14-Eicosatetraenoic Acid[c] (C20:4)	Arachidonic Acid	0.56 ± 0.25

[a] Fatty acid concentrations are expressed as free fatty acids. To convert to the equivalent triglyceride or methyl ester (FAME) concentration, see Reference 5.

[b] Each reference concentration value, expressed as a mass fraction of the material as received, is an equally weighted mean of results from an interlaboratory comparison exercise among the laboratories listed in Appendix A. The uncertainty in the reference value is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO Guide [1]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % level confidence for each analyte. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendix B.

[c] This is the primary component contributing to the measured value. There may be low levels of other unsaturated fatty acids that coeluted with the principal component and contributed to the signal measured.

Table 4. Reference Concentrations for Proximates and Calories[a]

NOTE: These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification and/or analyses have not been performed at NIST. These reference values should be useful for comparison with results obtained using similar procedures (i.e., AOAC methods).

Constituent	Mass Fraction (%)
Solids	40.5 ± 2.6
Ash	3.21 ± 0.21
Extractable Fat	21.0 ± 1.4
Fat as Sum of Fatty Acids[b]	19.7 ± 2.1
Protein	14.9 ± 1.0
Carbohydrates[c]	1.77 ± 0.19
Calories[d]	252 ± 17 kcal/100 g

[a] Each reference concentration value, expressed as a mass fraction of the material as received, is an equally weighted mean of results from an interlaboratory comparison exercise among the laboratories listed in Appendix A. The uncertainty in the reference value is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO Guide [1]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % level confidence for each analyte. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendix B.

[b] This value is the sum of the individual fatty acids as triglycerides.

[c] This value for carbohydrates is from the measured mass fraction of sucrose (see Table 5). Many of the laboratories looked for other sugars, but in most cases levels of these were below the limits of quantitation. If carbohydrates were calculated by summing the mean mass fractions of water, ash, protein, and extractable fat and subtracting that sum from 100, the result would be 1.4 %, while the mean carbohydrate level reported by the laboratories was 2.5 %. In both of these cases, the uncertainty is larger than the mean.

[d] Note that the value for calories is the mean of individual caloric calculations from the NFPA round robin exercise. If the mean proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat, protein, and carbohydrate, respectively, the mean caloric content is 256 kcal/100 g if extractable fat is used and 244 kcal/100 g if fat from the sum of the fatty acids is used.

Table 5. Reference Concentrations for Selected Water-Soluble Vitamins and Sucrose[a]

NOTE: These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification and/or analyses have not been performed at NIST.

Constituent	Mass Fraction (mg/kg)
Vitamin B_1·HCl	2.16 ± 0.44
Vitamin B_2	2.00 ± 0.59
Vitamin B_6	1.30 ± 0.61
Vitamin B_{12}	0.006 ± 0.001
Niacin	36.3 ± 3.8
Pantothenic Acid	5.76 ± 0.65
Biotin	0.036 ± 0.011
	Mass Fraction (g/kg)
Sucrose	17.7 ± 1.9

[a] Each reference concentration value, expressed as a mass fraction of the material as received, is an equally weighted mean of results from an interlaboratory comparison exercise among the laboratories listed in Appendix A. The uncertainty in the reference value is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO Guide [1]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % level confidence for each analyte. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendix B.

Table 6. Reference Concentrations for Minerals and Trace Elements[a]

NOTE: These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification and/or analyses have not been performed at NIST.

Constituent	Mass Fraction (mg/kg)
Chloride	15200 ± 1100
Copper	0.93 ± 0.18
Magnesium	165 ± 12
Manganese	0.292 ± 0.044
Phosphorus	1540 ± 110
Potassium	2460 ± 160
Zinc	18.5 ± 1.6

[a] Each reference concentration value, expressed as a mass fraction of the material as received, is an equally weighted mean of results from an interlaboratory comparison exercise among the laboratories listed in Appendix A. The uncertainty in the reference value is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO Guide [1]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % level confidence for each analyte. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendix B.

Table 7. Information Concentrations for Additional Constituents[a]

NOTE: These concentrations are provided as information values only because the disagreement among the methods was greater than expected for reference values or because results were reported by a limited number of laboratories. The data for these information values are not of sufficient quality or quantity to adequately assign uncertainties.

Constituent	Mass Fraction (mg/kg)
Folic acid	0.012
Choline (ion)	580
Inositol	230
Boron	0.28
Iodine	0.24
Sulfur	1900

[a] Information values are the equally weighted means of results obtained by the laboratories listed in Appendix A reported on an "as received" basis. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendix B.

REFERENCES

[1] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed., ISO, Geneva, Switzerland, (1993): see also Taylor, B.N. and Kuyatt, C.E., "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results," NIST Technical Note 1297, U.S. Government Printing Office, Washington DC, (1994).

[2] Schiller, S.B. and Eberhardt, K.E., "Combining Data from Independent Chemical Analysis Methods," *Spectrochim. Acta*, **46B**, No. 12, pp. 1607-1613, (1991).

[3] Ellerbe, P., Meiselman, S., Sniegoski, L.T., Welch, M.J., and White V, E., "Determination of Serum Cholesterol by a Modification of the Isotope Dilution Mass Spectrometric Definitive Method," *Anal. Chem*, **61**, pp. 1718-1723, (1989).

[4] Hydrolysis procedure used: Method 43.235, *Official Methods of Analysis*, 13th Ed., AOAC, Gaithersburg, MD, (1980).

[5] Conversions between Free Fatty Acids and either Triglycerides or FAMEs: *AOAC Official Methods of Analysis* (1995), Supplement March 1997, Ch. 41, pp. 18B-C.

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: Telephone (301) 975-6776 (select "Certificates"), Fax (301) 926-4751, e-mail srminfo@nist.gov, or via the Internet http://ts.nist.gov/srm

APPENDIX A

Analysts at the laboratories listed below performed measurements that contributed to the value assignment of fatty acids, cholesterol, proximates, vitamins, and/or minerals in SRM 1546 Meat Homogenate.

Beech-Nut Nutrition Corporation, Canajoharie, NY
Campbell Soup Company, Camden, NJ
Covance, Inc., Madison, WI
Del Monte Foods Company, Walnut Creek, CA
Dionex Corporation, Salt Lake City, UT[1]
The Dial Corporation, Scottsdale, AZ
Food and Drug Administration, Washington, DC[2]
General Mills, Inc., Minneapolis, MN
Gerber Products Company, Fremont, MI
Hormel Foods Corporation, Austin, MN
Kraft Foods, Glenview, IL
Krueger Food Laboratories, Cambridge, MA
Lancaster Laboratories, Lancaster, PA
Nabisco, Inc., East Hanover, NJ
Nestlé USA, Dublin, OH
Novartis Nutrition Corporation, St. Louis Park, MN
Ralston Purina Company, St. Louis, MO
U.S. Department of Agriculture, Beltsville, MD
U.S. Department of Agriculture, Peoria, IL[1]
Woodson-Tenent Laboratories, Memphis, TN

[1] These laboratories are not part of the NFPA FIACS and performed analyses related only to the fat content of SRM 1546.

[2] This laboratory is not part of the NFPA FIACS and performed analyses for various inorganic constituents.

APPENDIX B

Methodological information reported by the collaborating laboratories (Appendix A) whose results were used for value assignment is summarized below. The number of laboratories using a particular method is provided in parentheses.

Proximates, Cholesterol, Calories, Nitrogen, and Sucrose

Solids	Moisture determined by mass loss after oven-drying: Forced-air oven (6) Vacuum oven (10) Microwave (1)
Ash	Mass loss after ignition in muffle furnace (17)
Extractable Fat	Acid digestion, ether extraction (9) Chloroform/methanol extraction (2) Soxhlet extraction (2) Pressurized fluid extraction (1) Supercritical fluid extraction (1)
Fat by Summation of Fatty Acids	Fatty acid quantitation by gas chromatography (9)
Nitrogen	Kjeldahl (11) Thermal conductivity (5) Autoanalyzer (1) Thermal neutron prompt gamma activation analysis (1)
Protein	Calculated; a factor of 6.25 was used to calculate protein from nitrogen results

Carbohydrates	Calculated; carbohydrate = solids - (protein + fat + ash)
Cholesterol	Gas chromatography (14) Gas chromatography/mass spectrometry (1)
Calories	Calculated; calories = 9(fat) + 4(protein) + 4(carbohydrate)
Sugars	Liquid chromatography - refractive index detection (10) Gas chromatography (1)

Water-Soluble Vitamins

Vitamin B_1·HCl	Microbiological (2) Digestion - fluorescence detection (6) Extraction - reversed-phase liquid chromatography - fluorescence detection (1) Extraction - ion pairing chromatography - fluorescence detection (1)
Vitamin B_2	Microbiological (2) Digestion - fluorescence detection (5) Extraction - reversed-phase liquid chromatography - fluorescence detection (3)
Vitamin B_6	Microbiological (7) Extraction - reversed-phase liquid chromatography - fluorescence detection (2)
Vitamin B_{12}	Microbiological (8)
Niacin	Microbiological (7) Acid digestion - absorption spectrophotometry (2)
Folic acid	Microbiological (6)
Pantothenic acid	Microbiological (8)
Biotin	Microbiological (6)
Choline	Acid digestion - absorption spectrophotometry (2) Microbiological (2)
Inositol	Microbiological (3)

Minerals and Trace Elements

Boron	Thermal neutron prompt gamma activation analysis (1)
Calcium	Flame atomic absorption spectrometry (7) Inductively coupled plasma atomic emission spectrometry (8) Direct current plasma atomic emission spectrometry (1)
Chloride	Thermal neutron prompt gamma activation analysis (1) Colorimetric titration (5) Electrochemical titration (5) Inductively coupled plasma atomic emission spectrometry (1) Mercury thiocyanate (1)
Copper	Flame atomic absorption spectrometry (8) Inductively coupled plasma atomic emission spectrometry (7) Direct current plasma atomic emission spectrometry (1)

Iodine	Colorimetric titration (1) Inductively coupled plasma atomic emission spectrometry (1)
Iron	Flame atomic absorption spectrometry (7) Inductively coupled plasma atomic emission spectrometry (8) Direct current plasma atomic emission spectrometry (1)
Magnesium	Flame atomic absorption spectrometry (7) Inductively coupled plasma atomic emission spectrometry (8) Direct current plasma atomic emission spectrometry (1)
Manganese	Flame atomic absorption spectrometry (6) Inductively coupled plasma atomic emission spectrometry (7) Direct current plasma atomic emission spectrometry (1)
Phosphorus	Absorption spectrophotometry (4) Inductively coupled plasma atomic emission spectrometry (8) Colorimetric titration (1) Molybdovanadate with perchloric acid (1)
Potassium	Flame atomic absorption spectrometry (7) Inductively coupled plasma atomic emission spectrometry (8) Direct current plasma atomic emission spectrometry (1) Thermal neutron prompt gamma activation analysis (1)
Sodium	Flame atomic absorption spectrometry (2) Flame atomic emission spectrometry (6) Inductively coupled plasma atomic emission spectrometry (7) Direct current plasma atomic emission spectrometry (1) Thermal neutron prompt gamma activation analysis (1)
Sulfur	Thermal neutron prompt gamma activation analysis (1)
Zinc	Flame atomic absorption spectrometry (6) Inductively coupled plasma atomic emission spectrometry (6) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma mass spectrometry (2)